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(54) Title: OVARIAN TUMOR-ASSOCIATED SEQUENCES

(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer, are disclosed. Compositions may comprise one or more ovarian tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses an ovarian tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as ovarian cancer. Diagnostic methods based on detecting an ovarian tumor protein, or mRNA encoding such a protein, in a sample are also provided.

OVARIAN TUMOR-ASSOCIATED SEQUENCES

TECHNICAL FIELD

The present invention relates generally to therapy and diagnosis of
5 cancer, such as ovarian cancer. The invention is more specifically related to
polypeptides comprising at least a portion of an ovarian tumor protein, and to
polynucleotides encoding such polypeptides. Such polypeptides and polynucleotides
may be used in vaccines and pharmaceutical compositions for prevention and treatment
of cancers such as ovarian cancer, and for the diagnosis and monitoring of such cancers.

10 BACKGROUND OF THE INVENTION

Ovarian cancer is a significant health problem for women in the United
States and throughout the world. Although advances have been made in detection and
therapy of this cancer, no vaccine or other universally successful method for prevention
or treatment is currently available. Management of the disease currently relies on a
15 combination of early diagnosis and aggressive treatment, which may include one or
more of a variety of treatments such as surgery, radiotherapy, chemotherapy and
hormone therapy. The course of treatment for a particular cancer is often selected based
on a variety of prognostic parameters, including an analysis of specific tumor markers.
However, the use of established markers often leads to a result that is difficult to
20 interpret, and high mortality continues to be observed in many cancer patients.

Immunotherapies have the potential to substantially improve cancer
treatment and survival. Such therapies may involve the generation or enhancement of
an immune response to an ovarian carcinoma antigen. However, to date, relatively few
ovarian carcinoma antigens are known and the generation of an immune response
25 against such antigens has not been shown to be therapeutically beneficial.

Accordingly, there is a need in the art for improved methods for
identifying ovarian tumor proteins and for using such proteins in the therapy of ovarian
cancer. The present invention fulfills these needs and further provides other related
advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods for the diagnosis and therapy of cancer, such as ovarian cancer. In one aspect, the present invention provides polypeptides comprising at least a portion of an ovarian tumor protein, or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises a sequence that is encoded by a polynucleotide sequence selected from the group consisting of sequences recited in SEQ ID NOs: 1-1502), variants of such sequences and complements of such sequences.

The present invention further provides polynucleotides that encode a polypeptide as described above, or a portion thereof (such as a portion encoding at least 15 amino acid residues of an ovarian tumor protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, vaccines are provided. Such vaccines comprise a polypeptide or polynucleotide as described above and a non-specific immune response enhancer.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to an ovarian tumor protein; and (b) a physiologically acceptable carrier.

Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Antigen presenting cells include dendritic cells, macrophages and B cells.

Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a non-specific immune response enhancer.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins.

Within related aspects, pharmaceutical compositions comprising a fusion
5 protein, or a polynucleotide encoding a fusion protein, in combination with a physiologically acceptable carrier are provided.

Vaccines are further provided, within other aspects, that comprise a fusion protein or a polynucleotide encoding a fusion protein in combination with a non-specific immune response enhancer.

10 Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as recited above. The patient may be afflicted with a cancer, in which case the methods provide treatment for the disease, or a patient considered at risk for such a disease may be treated prophylactically.

15 The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with an ovarian tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

20 Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for an ovarian tumor protein, comprising contacting T
25 cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of an ovarian tumor protein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expresses such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer in a patient, comprising (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody. The cancer may be ovarian cancer.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a)

contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes an ovarian tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes an ovarian tumor protein; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for the therapy and diagnosis of cancer, such as ovarian cancer. The compositions described herein may comprise ovarian tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (e.g., T cells). Polypeptides of the present invention generally comprise at least a portion (such as an immunogenic portion) of an ovarian tumor protein or a variant thereof. An "ovarian tumor protein" is a protein that is encoded by an ovarian tumor polynucleotide, as provided herein. An "ovarian tumor polynucleotide" is a polynucleotide that comprises, or is complementary to, a cDNA sequence that is present at a greater level in cDNA from ovarian tumor cells than in cDNA from non-ovarian tissue. Preferably, the level in ovarian tumor cDNA is at least two fold, more preferably at least five fold, greater than the level of expression in a non-ovarian tissue. Certain ovarian tumor proteins are tumor-specific (*i.e.*, are expressed at higher levels in ovarian tumors than in normal ovarian cells). Such proteins may be used as markers to detect or monitor ovarian cancer in patients. Other ovarian tumor proteins are present at comparable levels in both normal ovarian cells and ovarian tumor cells. These proteins may be ovary-tissue specific. Antibodies are generally immune system proteins, or antigen-binding fragments thereof, that are capable of binding to a polypeptide as described above. Antigen presenting cells include dendritic cells and macrophages that express a polypeptide as described above. T cells that may be employed within such compositions are generally T cells that are specific for a polypeptide as described above.

The present invention is based on the discovery of previously unknown human ovarian tumor proteins. Partial sequences of polynucleotides encoding specific tumor proteins are provided in SEQ ID NOs:1-1502.

OVARIAN TUMOR POLYNUCLEOTIDES

Any polynucleotide that encodes an ovarian tumor protein or a portion or other variant thereof as described herein is encompassed by the present invention. Preferred polynucleotides comprise at least 15 consecutive nucleotides, preferably at

least 30 consecutive nucleotides and more preferably at least 45 consecutive nucleotides, that encode a portion of an ovarian tumor protein. More preferably, a polynucleotide encodes an immunogenic portion of an ovarian tumor protein. Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes an ovarian tumor protein or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native ovarian tumor protein or a portion thereof.

The percent identity for two polynucleotide or polypeptide sequences may be readily determined by comparing sequences using computer algorithms well known to those of ordinary skill in the art, such as Megalign, using default parameters. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Optimal alignment of sequences for comparison may be conducted, for example, using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using

default parameters. Preferably, the percentage of sequence identity is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the window may comprise additions or deletions (*i.e.*, gaps) of 20 % or less, usually 5 to 15
5 %, or 10 to 12%, relative to the reference sequence (which does not contain additions or deletions). The percent identity may be calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window
10 size) and multiplying the results by 100 to yield the percentage of sequence identity.

Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native ovarian tumor protein (or a complementary sequence).
15 Suitable moderately stringent conditions include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

It will be appreciated by those of ordinary skill in the art that, as a result
20 of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences
25 provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

30 Polynucleotides may be prepared using any of a variety of techniques. For example, a polynucleotide may be identified using a PCR-based cDNA subtraction

approach. Briefly, such screens may be performed using the PCR-Select cDNA Subtraction method (Clontech). The tester cDNA library may be obtained using pooled mRNA from multiple ovarian metastatic tumors, and the driver library may be obtained from pooled mRNA from multiple non-ovarian normal tissues. Alternatively, a
5 microarray of cDNAs may be screened for tumor-associated expression. Such screens may be performed using a Synteni microarray (Palo Alto, CA) according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA* 93:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA* 94:2150-2155, 1997). Within yet another screen, polynucleotides may be amplified
10 from cDNA prepared from cells expressing the proteins described herein, such as ovarian tumor cells. Such polynucleotides may be amplified via polymerase chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion may be used to isolate a full length gene from a
15 suitable library (e.g., an ovarian tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or genomic) is screened using one or more polynucleotide probes or primers suitable for amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for
20 obtaining introns and extending 5' sequences.

For hybridization techniques, a partial sequence may be labeled (e.g., by nick-translation or end-labeling with ³²P) using well known techniques. A bacterial or bacteriophage library is then screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (see
25 Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and
30 partial sequences may be generated to identify one or more overlapping clones. The complete sequence may then be determined using standard techniques, which may

involve generating a series of deletion clones. The resulting overlapping sequences are then assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining
5 a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target
10 sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia et al., *Nucl. Acids Res.* 16:8186, 1988), which uses restriction enzymes to generate a fragment in the
15 known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of
20 amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer,
25 which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom et al., *PCR Methods Applic. 1*:111-19, 1991) and walking PCR (Parker et al., *Nucl. Acids Res.* 19:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

30 In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as

that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence.

Certain nucleic acid sequences of cDNA molecules encoding portions of
5 ovarian tumor proteins are provided in SEQ ID NOs:1-1502. These polynucleotides were isolated by PCR-based subtraction. This technique serves to normalize differentially expressed cDNAs, facilitating the recovery of rare transcripts, and also has the advantage of permitting enrichment of cDNAs with small amounts of polyA RNA material and without multiple rounds of hybridization. In one subtraction, a pool of
10 four ovary metastatic omentum mRNAs was used to generate the tester library. This library was subtracted with driver mRNA, pooled from brain, pancreas, bone marrow, lung, heart, kidney, liver and trachea. In a second subtraction, the tester library was generated from a pool of primary ovary tumor mRNAs, including an endometroid adenocarcinoma, a germ cell tumor, a papillary serous adenocarcinoma and a clear cell
15 carcinoma, and subtracted with the driver library discussed above. PCR-amplified pools were generated from the subtracted libraries and clones corresponding to SEQ ID NOs:1-1502 were sequenced.

Polynucleotide variants may generally be prepared by any method known in the art, including chemical synthesis by, for example, solid phase phosphoramidite
20 chemical synthesis. Modifications in a polynucleotide sequence may also be introduced using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (*see* Adelman et al., *DNA* 2:183, 1983). Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding an ovarian tumor protein, or portion thereof, provided that the DNA is incorporated into a
25 vector with a suitable RNA polymerase promoter (such as T7 or SP6). Certain portions may be used to prepare an encoded polypeptide, as described herein. In addition, or alternatively, a portion may be administered to a patient such that the encoded polypeptide is generated *in vivo* (*e.g.*, by transfecting antigen-presenting cells, such as dendritic cells, with a cDNA construct encoding an ovarian tumor polypeptide, and
30 administering the transfected cells to the patient).

A portion of a sequence complementary to a coding sequence (*i.e.*, an antisense polynucleotide) may also be used as a probe or to modulate gene expression. cDNA constructs that can be transcribed into antisense RNA may also be introduced into cells or tissues to facilitate the production of antisense RNA. An antisense
5 polynucleotide may be used, as described herein, to inhibit expression of a tumor protein. Antisense technology can be used to control gene expression through triple-helix formation, which compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors or regulatory molecules (*see* Gee et al., *In* Huber and Carr, *Molecular and Immunologic Approaches*, Futura Publishing Co.
10 (Mt. Kisco, NY; 1994)). Alternatively, an antisense molecule may be designed to hybridize with a control region of a gene (*e.g.*, promoter, enhancer or transcription initiation site), and block transcription of the gene; or to block translation by inhibiting binding of a transcript to ribosomes.

A portion of a coding sequence or of a complementary sequence may
15 also be designed as a probe or primer to detect gene expression. Probes may be labeled with a variety of reporter groups, such as radionuclides and enzymes, and are preferably at least 10 nucleotides in length, more preferably at least 20 nucleotides in length and still more preferably at least 30 nucleotides in length. Primers, as noted above, are preferably 22-30 nucleotides in length.

20 Any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl- methyl-, thio- and
25 other modified forms of adenine, cytidine, guanine, thymine and uridine.

Nucleotide sequences as described herein may be joined to a variety of other nucleotide sequences using established recombinant DNA techniques. For example, a polynucleotide may be cloned into any of a variety of cloning vectors, including plasmids, phagemids, lambda phage derivatives and cosmids. Vectors of
30 particular interest include expression vectors, replication vectors, probe generation vectors and sequencing vectors. In general, a vector will contain an origin of replication

functional in at least one organism, convenient restriction endonuclease sites and one or more selectable markers. Other elements will depend upon the desired use, and will be apparent to those of ordinary skill in the art.

Within certain embodiments, polynucleotides may be formulated so as to permit entry into a cell of a mammal, and expression therein. Such formulations are particularly useful for therapeutic purposes, as described below. Those of ordinary skill in the art will appreciate that there are many ways to achieve expression of a polynucleotide in a target cell, and any suitable method may be employed. For example, a polynucleotide may be incorporated into a viral vector such as, but not limited to, adenovirus, adeno-associated virus, retrovirus, or vaccinia or other pox virus (e.g., avian pox virus). The polynucleotides may also be administered as naked plasmid vectors. Techniques for incorporating DNA into such vectors are well known to those of ordinary skill in the art. A retroviral vector may additionally transfer or incorporate a gene for a selectable marker (to aid in the identification or selection of transduced cells) and/or a targeting moiety, such as a gene that encodes a ligand for a receptor on a specific target cell, to render the vector target specific. Targeting may also be accomplished using an antibody, by methods known to those of ordinary skill in the art.

Other formulations for therapeutic purposes include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A preferred colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*i.e.*, an artificial membrane vesicle). The preparation and use of such systems is well known in the art.

25 OVARIAN TUMOR POLYPEPTIDES

Within the context of the present invention, polypeptides may comprise at least an immunogenic portion of an ovarian tumor protein or a variant thereof, as described herein. As noted above, an "ovarian tumor protein" is a protein that is expressed by ovarian tumor cells. Proteins that are ovarian tumor proteins may also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with ovarian cancer. Polypeptides as described herein may be of any length. Additional

sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of an ovarian tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native ovarian tumor protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

- As noted above, a composition may comprise a variant of a native ovarian tumor protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native ovarian tumor protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.
- Polypeptide variants preferably exhibit at least about 70%, more preferably at least about 90% and most preferably at least about 95% identity to the native polypeptide. The percent identity may be determined as described above. Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a

preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydrophobic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells, such as mammalian or plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means, using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.*

85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

5 Within certain specific embodiments, a polypeptide may be a fusion protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, 10 or may assist in expressing the protein (an expression enhancer) at higher yields than the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which 15 facilitate purification of the protein.

 Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide 20 components may be assembled separately, and ligated into an appropriate expression vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of 25 both component polypeptides.

 A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. 30 Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a

secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as linkers include those disclosed in Maratea et al., *Gene* 40:39-46, 1985; Murphy et al., *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Also provided are fusion proteins that comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably, the immunogenic protein is capable of eliciting a recall response. Examples of such proteins include tetanus, tuberculosis and hepatitis proteins (*see, e.g.,* Stoute et al., *New Engl. J. Med.* 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza B* (WO 91/18926). Preferably, a protein D derivative comprises approximately the first third of the protein (*e.g.,* the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen present cells. Other

fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine amidase known as amidase LYTA (encoded by the *LytA* gene; *Gene* 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (see *Biotechnology* 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

25

BINDING AGENTS

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to an ovarian tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to an ovarian tumor protein if it reacts at a detectable level (within, for example, an ELISA) with an ovarian tumor protein, and does not react detectably with unrelated

proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the
5 concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients
10 with and without a cancer, such as ovarian cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to an ovarian tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the
15 cancer. To determine whether a binding agent satisfies this requirement, biological samples (e.g., blood, sera, leukophoresis, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the
20 disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component,
25 an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation
30 of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of

recombinant antibodies. In one technique, an immunogen comprising the polypeptide is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from

the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

5 Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested
10 by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides
15 include ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , and ^{212}Bi . Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin, *Shigella* toxin, and pokeweed antiviral protein.

20 A therapeutic agent may be coupled (*e.g.*, covalently bonded) to a suitable monoclonal antibody either directly or indirectly (*e.g.*, via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulphydryl group, on one may be capable of reacting with a carbonyl-
25 containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker
30 group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical

reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, 5 sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spittler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler et al.). 15

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used. 25

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for 30

radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for an ovarian tumor protein. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the CEPRATE™ system, available from CellPro Inc., Bothell WA (see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with an ovarian tumor polypeptide, polynucleotide encoding an ovarian tumor polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, an ovarian tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for an ovarian tumor polypeptide if the T cells kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen et al., *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (e.g., by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with an ovarian tumor polypeptide (100 ng/ml - 100 µg/ml, preferably 200 ng/ml - 25 µg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (e.g., TNF or IFN-γ) is indicative of T cell activation (see Coligan et al., *Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to an ovarian tumor polypeptide, polynucleotide or polypeptide-expressing APC may be CD4⁺ and/or CD8⁺. Ovarian tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are derived from a patient, or from a related or unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, CD4⁺ or CD8⁺ T cells that proliferate in response to an ovarian tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to an ovarian tumor polypeptide (e.g., a short peptide corresponding to an immunogenic portion of such a polypeptide) with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize an ovarian tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of an

ovarian tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution. Following expansion, the cells may be administered back to the patient as described, for example, by Chang et al., *Crit. Rev. Oncol. Hematol.* 22:213, 1996.

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PHARMACEUTICAL COMPOSITIONS AND VACCINES

Within certain aspects, polypeptides, polynucleotides, T cells and/or binding agents described herein may be incorporated into pharmaceutical compositions or immunogenic compositions (*i.e.*, vaccines). Pharmaceutical compositions comprise one or more such compounds and a physiologically acceptable carrier. Vaccines comprise one or more such compounds and a non-specific immune response enhancer. A non-specific immune response enhancer may be any substance that enhances an immune response to an exogenous antigen. Examples of non-specific immune response enhancers include adjuvants, biodegradable microspheres (*e.g.*, polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.*, Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Vaccines may be designed to generate antibody immunity and/or cellular immunity such as that arising from CTL or CD4+ T cells.

20 Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine. Polypeptides may, but need not, be conjugated to other macromolecules as described, for example, within US Patent Nos. 4,372,945 and 4,474,757. Pharmaceutical compositions and vaccines may generally be used for prophylactic and therapeutic purposes.

25 A pharmaceutical composition or vaccine may contain a polynucleotide encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. Such a polynucleotide may comprise DNA, RNA, a modified nucleic acid or a DNA/RNA hybrid. As noted above, a polynucleotide may be present

within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner et al., *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld et al., *Science* 252:431-434, 1991; Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler et al., *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman et al., *Circulation* 88:2838-2848, 1993; and Guzman et al., *Cir. Res.* 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a vaccine may comprise both a polynucleotide and a polypeptide component. Such vaccines may provide for an enhanced immune response.

It will be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (e.g., salts of primary, secondary and tertiary amines and basic amino acids) and

inorganic bases (e.g., sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647; 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252.

Such compositions may also comprise buffers (e.g., neutral buffered saline or phosphate buffered saline), carbohydrates (e.g., glucose, mannose, sucrose or dextran), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives. Alternatively, compositions of the present invention may be formulated as a lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of non-specific immune response enhancers may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as, for example, Freund's

Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars;
5 cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type.
10 High levels of Th1-type cytokines (e.g., IFN- γ , TNF- α , IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-
15 type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

20 Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Ribi ImmunoChem Research Inc. (Hamilton, MT; see US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing
25 oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555 and WP 99/33488. Immunostimulatory DNA sequences are also described, for example, by Sato et al., *Science* 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila, United States), which may be
30 used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as

the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and
5 tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Ribi ImmunoChem Research Inc., Hamilton, MT), RC-529
10 (Ribi ImmunoChem Research Inc., Hamilton, MT) and Aminoalkyl glucosaminide 4-phosphates (AGPs).

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as
15 part of a sustained release formulation (*i.e.*, a formulation such as a capsule or sponge that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes et al., *Vaccine* 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-
20 release formulations may contain a polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of
25 active component release. Such carriers include microparticles of poly(lactide-co-glycolide), as well as polyacrylate, latex, starch, cellulose and dextran. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see*
30 *e.g.*, U.S. Patent No. 5, 151, 254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained

release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature* 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med.* 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see* Zitvogel et al., *Nature Med.* 4:594-600, 1998).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of

cytokines such as GM-CSF, IL-4, IL-13 and/or TNF α to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNF α ,
5 CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized phenotypes. However, this nomenclature should not be construed to exclude all
10 possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fc γ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high expression of cell surface molecules responsible for T cell activation such as class I and
15 class II MHC, adhesion molecules (e.g., CD54 and CD11) and costimulatory molecules (e.g., CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding an ovarian tumor protein (or portion or other variant thereof) such that the ovarian tumor polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such
20 transfection may take place *ex vivo*, and a composition or vaccine comprising such transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed
25 using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi et al., *Immunology and cell Biology* 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the ovarian tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g.,
30 vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help

(e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

CANCER THERAPY

In further aspects of the present invention, the compositions described herein may be used for immunotherapy of cancer, such as ovarian cancer. Within such methods, pharmaceutical compositions and vaccines are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor.

Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. Administration may be by any suitable method, including administration by intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as CD8⁺ cytotoxic T lymphocytes and CD4⁺ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive immunotherapy.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above, immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow *in vivo* and to survive long term in substantial numbers by repeated

stimulation with antigen supplemented with IL-2 (*see*, for example, Cheever et al., *Immunological Reviews* 157:177, 1997).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that leads to an improved clinical outcome (*e.g.*, more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose ranges from about 1 μ g to 5 mg, preferably 100 μ g to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical

outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to an ovarian tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using samples obtained from a patient before and after treatment.

METHODS FOR DETECTING CANCER

In general, a cancer may be detected in a patient based on the presence of one or more ovarian tumor proteins and/or polynucleotides encoding such proteins in a biological sample (such as blood, sera, urine and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as ovarian cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, an ovarian tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a

binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length ovarian tumor proteins and portions thereof to which the binding agent binds, as described above.

The solid support may be any material known to those of ordinary skill in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 μ g, and preferably about 100 ng to about 1 μ g, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports
5 having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.,* Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay.
10 This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a
15 different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically
20 blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact
25 time (*i.e.,* incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with ovarian cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide. Those of ordinary skill in the art will recognize that the time necessary to achieve
30 equilibrium may be readily determined by assaying the level of binding that occurs over

a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of a cancer, such as ovarian cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity)

that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered
5 positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or
10 strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of
15 bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the
20 presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a
25 positive signal in the two-antibody sandwich assay, in the format discussed above. Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological
30 sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use
5 ovarian tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such ovarian tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with an ovarian tumor protein in a biological sample.
10 Within certain methods, a biological sample comprising CD4⁺ and/or CD8⁺ T cells isolated from a patient is incubated with an ovarian tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated
15 T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with Mtb-81 or Mtb-67.2 polypeptide (*e.g.*, 5 - 25 µg/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of ovarian tumor polypeptide to serve as a
20 control. For CD4⁺ T cells, activation is preferably detected by evaluating proliferation of the T cells. For CD8⁺ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

25 As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding an ovarian tumor protein in a biological sample. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of an ovarian tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific
30 for (*i.e.*, hybridizes to) a polynucleotide encoding the ovarian tumor protein. The amplified cDNA is then separated and detected using techniques well known in the art,

such as gel electrophoresis. Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding an ovarian tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

5 To permit hybridization under assay conditions, oligonucleotide primers and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding an ovarian tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably,
10 oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous
15 nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule having a sequence recited in SEQ ID NOs:1-1502. Techniques for both PCR based assays and hybridization assays are well known in the art (*see*, for example, Mullis et al., *Cold Spring Harbor Symp. Quant. Biol.*, 51:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

20 One preferred assay employs RT-PCR, in which PCR is applied in conjunction with reverse transcription. Typically, RNA is extracted from a biological sample such as a biopsy tissue and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification
25 may be performed on biological samples taken from a test patient and from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered
30 positive.

In another embodiment, ovarian tumor proteins and polynucleotides encoding such proteins may be used as markers for monitoring the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the level of polypeptide detected by the binding agent increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide either remains constant or decreases with time.

10 Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

15 As noted above, to improve sensitivity, multiple ovarian tumor protein markers may be assayed within a given sample. It will be apparent that binding agents specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that result in optimal sensitivity. In addition, or alternatively, assays for tumor proteins
20 provided herein may be combined with assays for other known tumor antigens.

DIAGNOSTIC KITS

The present invention further provides kits for use within any of the
25 above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically binds to an ovarian tumor protein. Such antibodies or fragments may be provided attached to a support material,
30 as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain

a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding an ovarian tumor protein in a biological sample. Such kits generally comprise
5 at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding an ovarian tumor protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding an ovarian
10 tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE

Identification of Ovarian Tumor Protein cDNAs

5 This Example illustrates the identification of cDNA molecules encoding ovarian tumor proteins.

 Ovarian tumor polynucleotides were isolated by PCR-based subtraction. PolyA mRNA was prepared from ovary metastatic tumor tissues, ovary primary tumor tissues and normal tissues. Three cDNA libraries were constructed, PCR-subtracted
10 and analyzed. One library was constructed using a pool of four ovary metastatic omentum mRNAs (referred to herein as ovarian metastatic subtraction library or OvMS library). Two libraries were constructed using a pool of primary ovary tumor mRNAs, including an endometroid adenocarcinoma, a germ cell tumor, a papillary serous adenocarcinoma and a clear cell carcinoma (referred to as primary ovarian tumor
15 subtraction libraries or PrOvS1 and PrOvS2). PrOvS1 and PrOvS2 libraries were constructed from different PCR amplified pools from the same initial subtraction. Eight normal tissues were represented in the driver mRNA, including brain, pancreas, bone marrow, lung, heart, kidney, liver and trachea. PolyA mRNA

 cDNA synthesis, hybridization and PCR amplification were performed
20 according to Clontech's user manual (PCR-Select cDNA Subtraction), with the following changes: 1) cDNA was restricted with a mixture of enzymes, including MscI, PvuII, StuI and DraI, instead of the single enzyme RsaI; and 2) the ration of driver to tester cDNA was increased in the hybridization steps (to 76:1) to give a more stringent subtraction. To analyze the efficiency of the subtraction, housekeeping genes such as
25 actin and GADPH were PCR amplified from dilutions of subtracted as well as unsubtracted PCR samples. Furthermore, the complexity and redundancy of each library was characterized by sequencing 96 clones from each of the PCR subtraction libraries (OvMS, PrOvS1 and PrOvS2). These analyses indicated that the libraries are enriched for genes overexpressed in ovary tissue and ovary tumor samples.

30 Following PCR amplification, the cDNAs were cloned into the pCR2.1-TOPO plasmid vector (Invitrogen). 1502 sequences were obtained from the OvMS,

PrOvS1 and PrOvS2 libraries. These sequences are provided herein as SEQ ID NOs:1-1502.

From the foregoing it will be appreciated that, although specific
5 embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

1. An isolated polypeptide comprising at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502.
2. A polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1-1502.
3. An isolated polynucleotide encoding at least 15 amino acid residues of an ovarian tumor protein comprising an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1-1502.
4. A polynucleotide encoding an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1-1502.
5. An isolated polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1-1502.
6. An isolated polynucleotide comprising a sequence that hybridizes to a sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences, under moderately stringent conditions.

7. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 3-6.

8. An expression vector comprising a polynucleotide according to any one of claims 3-6.

9. A host cell transformed or transfected with an expression vector according to claim 8.

10. An expression vector comprising a polynucleotide according to claim 7.

11. A host cell transformed or transfected with an expression vector according to claim 10.

12. A pharmaceutical composition comprising a polypeptide according to claim 1, in combination with a physiologically acceptable carrier.

13. A vaccine comprising a polypeptide according to claim 1, in combination with a non-specific immune response enhancer.

14. A vaccine according to claim 13, wherein the non-specific immune response enhancer is an adjuvant.

15. A vaccine according to claim 13, wherein the non-specific immune response enhancer induces a predominantly Type I response.

16. A pharmaceutical composition comprising a polynucleotide according to any one of claims 3-6, in combination with a physiologically acceptable carrier.

17. A vaccine comprising a polynucleotide according to any one of claims 3-6, in combination with a non-specific immune response enhancer.

18. A vaccine according to claim 17, wherein the non-specific immune response enhancer is an adjuvant.

19. A vaccine according to claim 17, wherein the non-specific immune response enhancer induces a predominantly Type I response.

20. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to an ovarian tumor protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences.

21. A pharmaceutical composition comprising an antibody or fragment thereof according to claim 20, in combination with a physiologically acceptable carrier.

22. A pharmaceutical composition comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a pharmaceutically acceptable carrier or excipient.

23. A pharmaceutical composition according to claim 22, wherein the antigen presenting cell is a dendritic cell or a macrophage.

24. A vaccine comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a non-specific immune response enhancer.

25. A vaccine according to claim 24, wherein the non-specific immune response enhancer is an adjuvant.

26. A vaccine according to claim 24, wherein the non-specific immune response enhancer induces a predominantly Type I response.

27. A vaccine according to claim 24, wherein the antigen-presenting cell is a dendritic cell.

28. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a polypeptide that comprises at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences, and thereby inhibiting the development of a cancer in the patient.

29. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a polynucleotide that encodes a polypeptide comprising at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences, and thereby inhibiting the development of a cancer in the patient.

30. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an antibody or antigen-binding fragment thereof that specifically binds to an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide

sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences, and thereby inhibiting the development of a cancer in the patient.

31. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences, and thereby inhibiting the development of a cancer in the patient.

32. A method according to claim 31, wherein the antigen-presenting cell is a dendritic cell.

33. A method according to any one of claims 28-31, wherein the cancer is ovarian cancer.

34. A fusion protein comprising at least one polypeptide according to claim 1.

35. A fusion protein according to claim 34, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.

36. A fusion protein according to claim 34, wherein the fusion protein comprises a T helper epitope that is not present within the native ovarian tumor protein.

37. A fusion protein according to claim 34, wherein the fusion protein comprises an affinity tag.
38. An isolated polynucleotide encoding a fusion protein according to claim 34.
39. A pharmaceutical composition comprising a fusion protein according to claim 34, in combination with a physiologically acceptable carrier.
40. A vaccine comprising a fusion protein according to claim 34, in combination with a non-specific immune response enhancer.
41. A vaccine according to claim 40, wherein the non-specific immune response enhancer is an adjuvant.
42. A vaccine according to claim 40, wherein the non-specific immune response enhancer induces a predominantly Type I response.
43. A pharmaceutical composition comprising a polynucleotide according to claim 38, in combination with a physiologically acceptable carrier.
44. A vaccine comprising a polynucleotide according to claim 38, in combination with a non-specific immune response enhancer.
45. A vaccine according to claim 44, wherein the non-specific immune response enhancer is an adjuvant.
46. A vaccine according to claim 44, wherein the non-specific immune response enhancer induces a predominantly Type I response.

47. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a pharmaceutical composition according to claim 39 or claim 43.

48. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a vaccine according to claim 40 or claim 44.

49. A method for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) polynucleotides recited in any one of SEQ ID NOs:1-1502; and

(ii) complements of the foregoing polynucleotides;

wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

50. A method according to claim 49, wherein the biological sample is blood or a fraction thereof.

51. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated according to the method of claim 49.

52. A method for stimulating and/or expanding T cells specific for an ovarian tumor protein, comprising contacting T cells with one or more of:

(i) a polypeptide that comprises at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein

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comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences;

(ii) a polynucleotide encoding such a polypeptide; and/or

(iii) an antigen presenting cell that expresses such a polypeptide;

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

53. An isolated T cell population, comprising T cells prepared according to the method of claim 52.

54. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population according to claim 53.

55. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) a polypeptide that comprises at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences;

(ii) a polynucleotide encoding such a polypeptide; and

(iii) an antigen-presenting cell that expresses such a polypeptide, such that T cells proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

56. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) a polypeptide that comprises at least an immunogenic portion of an ovarian tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502, or a complement of any of the foregoing sequences;

(ii) a polynucleotide encoding such a polypeptide; and

(iii) an antigen-presenting cell that expresses such a polypeptide; such that T cells proliferate;

(b) cloning at least one proliferated cell; and

(c) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a cancer in the patient.

57. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with a binding agent that binds to an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(i) polynucleotides recited in any one of SEQ ID NOs:1-1502; and

(ii) complements of the foregoing polynucleotides;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(c) comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

58. A method according to claim 57, wherein the binding agent is an antibody.

59. A method according to claim 58, wherein the antibody is a monoclonal antibody.

60. A method according to claim 57, wherein the cancer is ovarian cancer.

61. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502 or a complement of any of the foregoing polynucleotides;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polypeptide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

62. A method according to claim 61, wherein the binding agent is an antibody.

63. A method according to claim 62, wherein the antibody is a monoclonal antibody.

64. A method according to claim 61, wherein the cancer is ovarian cancer.

65. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502 or a complement of any of the foregoing polynucleotides;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

66. A method according to claim 65, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

67. A method according to claim 65, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

68. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-1502 or a complement of any of the foregoing polynucleotides;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

69. A method according to claim 68, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

70. A method according to claim 68, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

71. A diagnostic kit, comprising:

(a) one or more antibodies according to claim 20; and

(b) a detection reagent comprising a reporter group.

72. A kit according to claim 71, wherein the antibodies are immobilized on a solid support.

73. A kit according to claim 72, wherein the solid support comprises nitrocellulose, latex or a plastic material.

74. A kit according to claim 71, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.

75. A kit according to claim 71, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

76. An oligonucleotide comprising 10 to 40 nucleotides that hybridize under moderately stringent conditions to a polynucleotide that encodes an ovarian tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs: 1-1502, or a complement of any of the foregoing polynucleotides.

77. A oligonucleotide according to claim 76, wherein the oligonucleotide comprises 10-40 nucleotides recited in any one of SEQ ID NOs: 1-1502.

78. A diagnostic kit, comprising:

- (a) an oligonucleotide according to claim 76; and
- (b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

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666

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240ttatatgaa gacaataaaa tcttgagttt atgtcagaa aaaaaaaaaa aaaaaaaaaa
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420cttaaaacnn atnccctga gggtttttta cctctggccc cngnaaccac cctttaaggg
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564

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536

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120aaatagccta aaatgaaaaa gaaacccgtt taacaagggt actaatcccc caacttcaa
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240ttattctgga aaatgccaat tgtacttaga caaaataagt taattctata tggttgtcca
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600ttaaaagaa nantctcttt ccttgttnt ttggngnagg gnancttgn cattaangtt
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778

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 600aangggggga nttgggcctt ttgtngcca aatngaacag ggttttttcc acatggtggg
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 745

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 120cttttcaagt tctgaactgg gaggtgactt tgagttcatg gatgatgcca acatgtgcat
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 420ggttggggcc cctaattaat ncttcntggn taattaacat taactcttga cttttaaagg
 480ggttcaactg gaataagcct tggggttttn ggaactgct tcnccgaan ccattcnaat
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 589

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103

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 120atgagaagtt ttaatgggct cccccaattg tctttttatt ttgggttatg acgatcatgt
 180ttgataaata caatgatagt ctctttccac gtgatgcttt ttgttgaaac tgaataaatt
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 544

<210> 571<211> 279<212> DNA<213> Homo sapiens
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 279

<210> 572<211> 555<212> DNA<213> Homo sapiens
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<210> 574<211> 345<212> DNA<213> Homo sapiens
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<210> 577<211> 552<212> DNA<213> Homo sapiens

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<210> 579<211> 563<212> DNA<213> Homo sapiens
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<210> 582<211> 380<212> DNA<213> Homo sapiens

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<210> 583<211> 498<212> DNA<213> Homo sapiens

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489

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 120tctgctttca cttaagtgct tggcccgcaa tactgtagga acaagcatga tcttgttact
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601

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101

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575

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319

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